Localization of Insulin-Like Growth Factor I and Inhibition of Coronary Smooth Muscle Cell Growth by Somatostatin Analogues in Human Coronary Smooth Muscle Cells

A Potential Treatment for Restenosis?

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Abstract In this study, we demonstrate, for the first time, the localization of insulin-like growth factor I (IGF-I) in de novo and restenotic human coronary atherectomy plaques by using immunocytochemical techniques. Smooth muscle cells (SMCs) exhibiting the synthetic phenotype contained a statistically significant higher concentration of IGF-I than SMCs of the contractile phenotype or SMCs from normal coronary arteries. In addition, we provide data to suggest that the long-acting somatostatin analogues octreotide and angiopeptin inhibit IGF-I– and basic fibroblast growth factor (b-FGF)–induced human coronary artery SMC proliferation. Platelet-derived growth factor (PDGF)-stimulated cultures were minimally affected by the addition of octreotide but were significantly inhibited by angiopeptin. All three growth factors stimulated SMC migration in a dose-dependent manner. The somatostatin analogues tested had no effect on growth factor–stimulated SMC migration. Our data suggest that by reducing SMC proliferation, somatostatin analogues may have clinical usefulness in reducing the high incidence of restenosis observed after percutaneous transluminal coronary artery interventions. (*Circulation*. 1994;89:1511-1517.)

The major limitation of percutaneous transluminal coronary artery interventions remains the high restenosis rate, occurring in as many as 57% of patients.[1] Despite advances in catheter-based technology such as directional, rotational, or excimer laser atherectomy, the recurrence rate has not been reduced.[2,3] A major breakthrough in understanding the pathophysiological processes that determine restenosis was recognition that a critical step in the chain of events is injury-induced activation of vascular smooth muscle cells (SMCs), resulting in cell proliferation and migration into the subintima.[4-6] These same cellular and molecular mechanisms may be responsible for the excessive SMC proliferation observed in transplant arteriopathy.[7]

Growth factors such as platelet-derived growth factor (PDGF), basic fibroblast growth factor (b-FGF), and insulin-like growth factor (IGF-I) have been implicated in the regulation of SMC proliferation and migration because all are potent SMC mitogens in vitro and induce SMC chemotaxis.[8-10] PDGF and b-FGF have been localized in human coronary atheroma by immunocytochemical techniques. Nikol and coworkers[11] recently demonstrated expression of transforming growth factor-β_1 (TGF-β) in human coronary primary atherosclerotic or restenotic lesions by in situ hybridization, suggesting that expression for TGF-β mRNA was significantly higher in restenotic compared with de novo lesions. These observations were corroborated by Rakugi et al,[12] who demonstrated the localization of TGF-β to discrete areas of mesenchymal-appearing intimal cells adjacent to foamy macrophages. The presence of IGF-I in human coronary lesions has not been reported.

IGF-I, b-FGF, and PDGF receptors belong to an expanded family of growth factor receptors, each sharing the common feature of a tyrosine kinase domain in the cytoplasmic portion of the molecule.[13] Binding of growth factors induces autophosphorylation of the β-subunit of the receptor and activation of tyrosine kinase. Deactivation of these growth factor receptors involves specific protein tyrosine phosphatases (PTPases).[14] Somatostatin, a growth-inhibitory peptide found throughout the body, activates PTPases and can inhibit the stimulatory effects of selected growth factors. Native somatostatin, however, has limited clinical use due to its extremely short half-life and overly broad range of inhibitory activities. Somatostatin analogues with longer half-lives, such as octreotide and angiopeptin, have been shown to have direct antiproliferative effects in a wide range of cell types in vitro and in vivo, and both agents have been used therapeutically in the treatment of gastrointestinal neoplasms, pituitary tumors, and prostatic cancer.[15]

Merimee[16] demonstrated that growth hormone–deficient dwarfs with diabetes who were followed for 25 years were free of atherosclerotic disease, suggesting a role for growth hormone in initiating or propagating

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atherogenesis. Others have noted acceleration of coronary artery disease and its complications in acromegalic patients.[17] Studies by Lundergan and coworkers[18] suggest that the absence of growth hormone or IGF-I, the mediator of the mitogenic actions of growth hormone, may attenuate restenosis. These researchers used angiopeptin and demonstrated inhibition of myointimal proliferation in response to balloon-induced endothelial cell injury in the rat carotid artery, rabbit aorta, and iliac and coronary arteries.[18] Also, hypophysectomy has been shown to inhibit arterial neointimal plaque formation in response to endothelial cell injury.[19] Santoian and coworkers[20] recently demonstrated that angiopeptin inhibits the development of intimal hyperplasia in swine coronary arteries after balloon injury.

The purpose of these studies was twofold. First, we determined whether IGF-I was present in human coronary atherectomy specimens. Second, we sought to determine whether octreotide and angiopeptin inhibited the growth-stimulatory and migratory effects of IGF-I, PDGF, and b-FGF in cultured human coronary SMCs.

Methods

Immunocytochemical Localization of IGF-I

Human coronary atheromatous tissue was obtained from patients undergoing directional coronary atherectomy in our cardiac catheterization laboratory as treatment for symptomatic coronary artery disease. Normal coronary arteries were obtained from three individuals between the ages of 18 and 26 years who had died accidentally in motor vehicle accidents. These controls were not matched for age, sex, or cardiovascular history to the atheromatous tissue donors. Atherectomy samples were fixed within 5 minutes after removal from the individual and placed in cold 5% acrolein, 0.1 mol/L Na cacodylate–HCl buffer (pH 7.4) for 30 minutes; washed in buffer 4×15 minutes; postfixed in 1% osmium tetroxide, 0.1 mol/L Na cacodylate–HCl buffer (pH 7.4); dehydrated in an ethanol series; infiltrated; and embedded in epoxy resin. Normal coronary arteries were fixed within 6 hours after death. Gold sections on nickel grids were oxidized for 5 minutes with 1% periodic acid, washed with water, and treated with 0.05% trypsin for 30 minutes at room temperature followed by a phosphate-buffered saline (PBS) wash. Grids were treated for 15 minutes with blocker (PBS containing 0.1 mol/L NaCl, 1% bovine serum albumin [BSA], 1% cold water fish gelatin [CWFG], and 1% nonfat dry milk [NDM]) followed by a 2-hour incubation at room temperature in affinity purified, polyclonal rabbit anti-human IGF-I antibodies (kind gift of Dr Jergen Zapf) diluted 1:200 in PBS plus the same additives as contained in the blocker. After 2×5-minute washes in PBS containing the same additives as used above, grids were washed 2×5 minutes in Tris-buffered saline (TBS) (TBS containing 0.1 mol/L NaCl, 1% BSA, 1% CWFG, and 1% NDM) followed by incubation for 1 hour at room temperature in goat anti-rabbit IgG secondary antibodies labeled with either 10 nm or 15 nm colloidal gold. Grids were washed 3×5 minutes in TBS followed by 3×5-minute washes in deionized water. Grids were examined and photographed in the electron microscope at 75 kV without poststaining. Controls for nonspecific labeling consisted of reaction with primary antibodies absorbed with IGF-I and reaction with secondary antibody without exposure to primary antibody.

Semiquantitation of the localization of IGF-I was done from representative electron micrographs at a final magnification of ×25 000 by two observers blind to the identity of the type of plaque (de novo or restenotic). Colloidal gold particles were counted, and final counts were expressed per unit area. Comparisons were made between synthetic SMCs[21] (sSMCs) and contractile SMCs (cSMCs) within the same plaque.

Preparation of SMC Cultures

For preparation of human coronary SMCs, recipient hearts explanted at the time of orthotopic heart transplantation were obtained, and a segment of the coronary artery was dissected under sterile conditions. The endothelium was first removed by scraping with a rubber policeman, and then the tissue was cut into uniform 1-mm^2 pieces using a McIlwain tissue chopper (Mickle Engineering, Surrey, UK). Each piece was placed in one well of a 2% gelatin–coated 96-well plate and covered with 100 μL of Dulbecco's modified Eagle's medium (DMEM) containing 20% fetal bovine serum (FBS), 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B. Cultures were placed in a humidified incubator containing 5% CO_2 at 37°C. Fresh media with 15% FBS were added by drops every 3 days until the tissue was just covered. By day 10, SMCs were radiating from the explant. Once cultures were established, tissue was removed, and cells within wells were allowed to reach confluence. Cells were expanded to 75-cm^2 flasks for continued growth in media with 15% FBS. SMCs were verified by typical "hill-and-valley" morphology, as well as by immunocytochemical staining with α-actin antiserum:[22] Cultures between passage levels 2 through 6 were used for these studies.

Proliferation and Cell Migration Studies

Single-cell suspensions of SMCs were plated in 24-well plates (5000 cells per well) in media with 2.5% FBS and allowed to adhere for 24 hours. After we washed the wells, media containing 2.5% FBS with the appropriate amount of growth factor alone or in combination with either octreotide (kind gift of Sandoz Pharmaceuticals) or angiopeptin (kind gift of Henri Beaufour Institute) were added to quadruplicate wells. Cells were counted on days 0, 2, 4, 6, 8, and 10. Day 0 represented 24 hours after initial seeding, ie, when growth factor and/or somatostatin analogue was added. Cells were enzymatically dissociated, and the entire well contents were counted using a model Z_F Coulter Counter (Coulter Electronics, Hialeah, Fla).

For thymidine incorporation experiments, cells were plated as above; 10 nmol/L IGF-I or 10 nmol/L b-FGF, and increasing concentrations of octreotide were added to wells along with 5 μCi per well of [^{3}H]thymidine. After an 18-hour incubation, wells were aspirated and washed three times. DNA was precipitated with trichloroacetic acid (TCA), solubilized with 0.3 N NaOH, and then counted in scintillation fluid.

Chemotaxis and chemokinesis assays were performed as previously described except that the assay duration was 12 hours and the optimal pore size for the porous polyvinyl- and pyrrolidone-free polycarbonate membrane (Nucleopore, Pleasanton, Calif) was 8 μm.[23] Each migration experiment was repeated a minimum of three times.

Statistical Analysis

The mean±SEM value for each set of studies was determined, and Student's t test was used to determine significance.

Results

IGF-I was localized in SMCs, macrophages, and foam cells, as well as in the extracellular matrix (ECM) of all plaques examined (n=10: 7 de novo and 3 restenotic). Localization was most intense in sSMCs and often associated with endoplasmic reticulum–derived vesicles and cell processes (Fig 1A). Specificity of the immunoreactivity for IGF-I was confirmed by the lack of labeling in an adjacent section of the same plaque reacted with IGF-I antibody absorbed with recombinant IGF-I (Fig 1B). The intensity of IGF-I localization was markedly reduced in quiescent cSMCs compared with

sSMCs in the same section of a given plaque (Fig 1C and 1D), but there was localization in the surrounding ECM. Mean colloidal gold particle counts per square micrometer for sSMCs and cSMCs were as follows: plaque 1—de novo (2.17±0.43 sSMCs, 0.42±0.06 cSMCs [$P<.01$]); plaque 2—de novo (2.89±0.78 sSMCs, 0.57±0.17 cSMCs [$P<.01$]); plaque 3—de novo (1.67±0.15 sSMCs, 0.51±0.20 cSMCs [$P<.05$]); plaque 4—restenotic (5.78±1.54 sSMCs, 0.51±0.20 cSMCs [$P<.001$]); plaque 5—restenotic (2.56±0.65 sSMCs, 0.28±0.05 cSMCs [$P<.05$]); and plaque 6—restenotic (1.78±0.29 sSMCs, 0.67±0.15 cSMCs [$P<.01$]). There was no significant difference between de novo and restenotic lesions because of the limited sample size. The mean colloidal gold particle count for SMCs of normal coronary arteries (n=3) is 0.08±0.02 counts/μm^2, which is significantly less than for SMCs of either synthetic ($P<.01$) or contractile phenotype ($P<.05$). The colloidal gold particle count in sections where the antibody was preabsorbed with IGF-I before exposure to the grids and in sections in which the primary antibody was omitted was 0.06±0.02 and 0.05±0.01 counts/μm^2, respectively.

The effects of IGF-I, b-FGF, and PDGF on human coronary SMC proliferation were assessed for 10 days. IGF-I induced a dose-dependent increase in SMC proliferation in the range of 1 to 100 nmol/L (data not shown). Time course studies, performed at 10 nmol/L, demonstrated a proliferative effect of IGF-I that began to plateau by days 8 through 10 (Fig 2a, top, open bars). b-FGF–stimulated cells demonstrated a pronounced proliferative response at day 2, which plateaued at days 6 through 8 (Fig 2a, middle, open bars). In a similar fashion, 10 nmol/L PDGF stimulated SMC proliferation, reaching maximal cell density on day 10 (Fig 2a, bottom, open bars).

SMC proliferation studies performed in the presence of IGF-I and octreotide are shown in the top panel of Fig 2a (filled bars). On days 2, 4, and 6, octreotide induced a 15% to 20% inhibition of IGF-I–stimulated SMC proliferation, which achieved statistical significance ($P<.01$). Addition of octreotide to b-FGF–stimulated SMCs resulted in a 40% to 45% inhibition of cell proliferation on days 6 and 8 (Fig 2a, middle, filled bars) ($P<.01$). In contrast, PDGF-stimulated cell growth was not inhibited by octreotide (Fig 2a, bottom, filled bars).

The effect of octreotide on rapidly proliferating SMCs treated with either IGF-I (10 nmol/L) alone or b-FGF (10 nmol/L) alone was tested, and the results are shown in Fig 2b. Tritiated thymidine incorporation in the IGF-I–stimulated cells was inhibited by 12±3% at a concentration of 1 nmol/L, 30±4% at a concentration of 10 nmol/L, and 43±3% at a concentration of 100 nmol/L, clearly demonstrating that octreotide inhibited DNA synthesis in a dose-dependent manner. The b-FGF–stimulated cells responded to octreotide with a 20±4%, 38±6%, and 61±4% decrease at a concentration of 1, 10, or 100 nmol/L, respectively.

Identical studies as performed with octreotide were performed using angiopeptin at a concentration of 30 nmol/L (Fig 2c). This dose was chosen based on clinical studies that demonstrated that threefold higher concentrations of angiopeptin are required to induce the identical clinical effect as octreotide.[15]

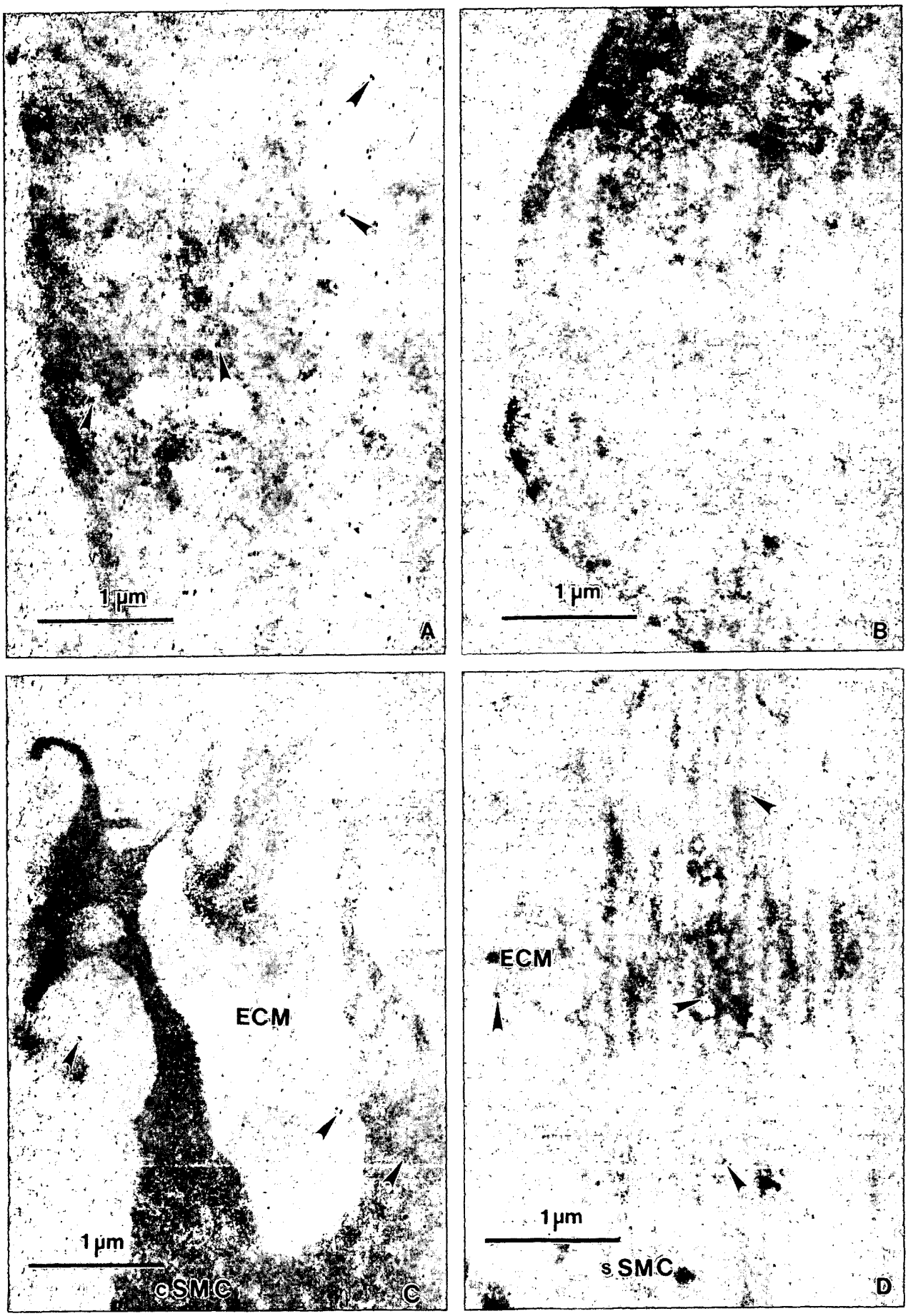
Interestingly, although octreotide showed no inhibitory effect on PDGF-stimulated cells, angiopeptin did show a 15% reduction in SMC growth compared with cultures stimulated with PDGF alone, and this reached statistical significance ($P<.05$).

Both IGF-I and b-FGF induced human coronary artery SMC migration in a dose-dependent manner that began to plateau at approximately 125 ng/mL. The maximal response to IGF-I was 28-fold greater than in the BSA control, whereas the b-FGF response was 37-fold greater than the control response. Chemotactic activity was inhibited by the addition of either IGF-I or b-FGF antibodies. Using the concentrations of each growth factor shown to be effective in the cell proliferation assays, 25 to 100 ng, the effect of each somatostatin analogue on inhibition of SMC migration was assessed. The simultaneous addition of octreotide or angiopeptin and IGF-I or b-FGF to SMCs resulted in apparently fewer cells migrating compared with the wells containing growth factor alone; however, this did not achieve statistical significance (data not shown).

Discussion

We describe, for the first time, the ultrastructural localization of immunoreactive IGF-I in coronary atherectomy plaques. Intense localization of IGF-I occurred within the endoplasmic reticulum and cell processes of SMCs exhibiting the synthetic phenotype. SMCs exhibiting the synthetic phenotype contained a significantly greater number of gold particles than SMCs exhibiting the contractile phenotype. This observation suggests that SMCs are synthesizing and secreting IGF-I and that IGF-I protein expression is a function of the proliferative state of the SMCs within the atheromatous plaque. IGF-I also localizes in the ECM; the origin of this IGF-I could be secretion by SMCs within the plaque. However, SMCs in the adjacent vessel wall could also be releasing IGF-I into the ECM. A third source of IGF-I could be serum as IGF-I has been shown to be taken up by vascular cells.[24] Immunocytochemical localization of IGF-I also occurs in foam cells and in the ECM of the fibrous cap. In agreement with the studies of Hansson and coworkers,[25] normal vessels did not show IGF-I immunoreactivity. These data should be interpreted with caution because of the time interval between fixation and the death of the donor, as well as the inability to match for variables such as age, sex, and cardiovascular history between the control and atheromatous tissues. Hansson et al demonstrated that injury to the tissue rapidly induced extensive blood vessel formation, and these new blood vessels transiently expressed IGF-I immunoreactivity. Our results also support the results of previous studies[26] showing that SMCs can produce their own growth factors, allowing growth to be sustained in an autocrine fashion.[27]

Because IGF-I–binding proteins (IGFBPs) regulate IGF-I bioactivity, their involvement in SMC proliferation must be considered. Elgin et al[28] purified IGFBP-1 from conditioned medium of porcine aortic SMCs and demonstrated that this IGFBP potentiated IGF-I–induced DNA synthesis and cell growth in these same cells. Studies by Cohick and Gockerman[29] demonstrated that porcine SMCs secrete IGFBP-2 and IGFBP-4. Insulin and forskolin induced a 41% increase in IGFBP-2 levels by radioimmunoassay in their cell sys-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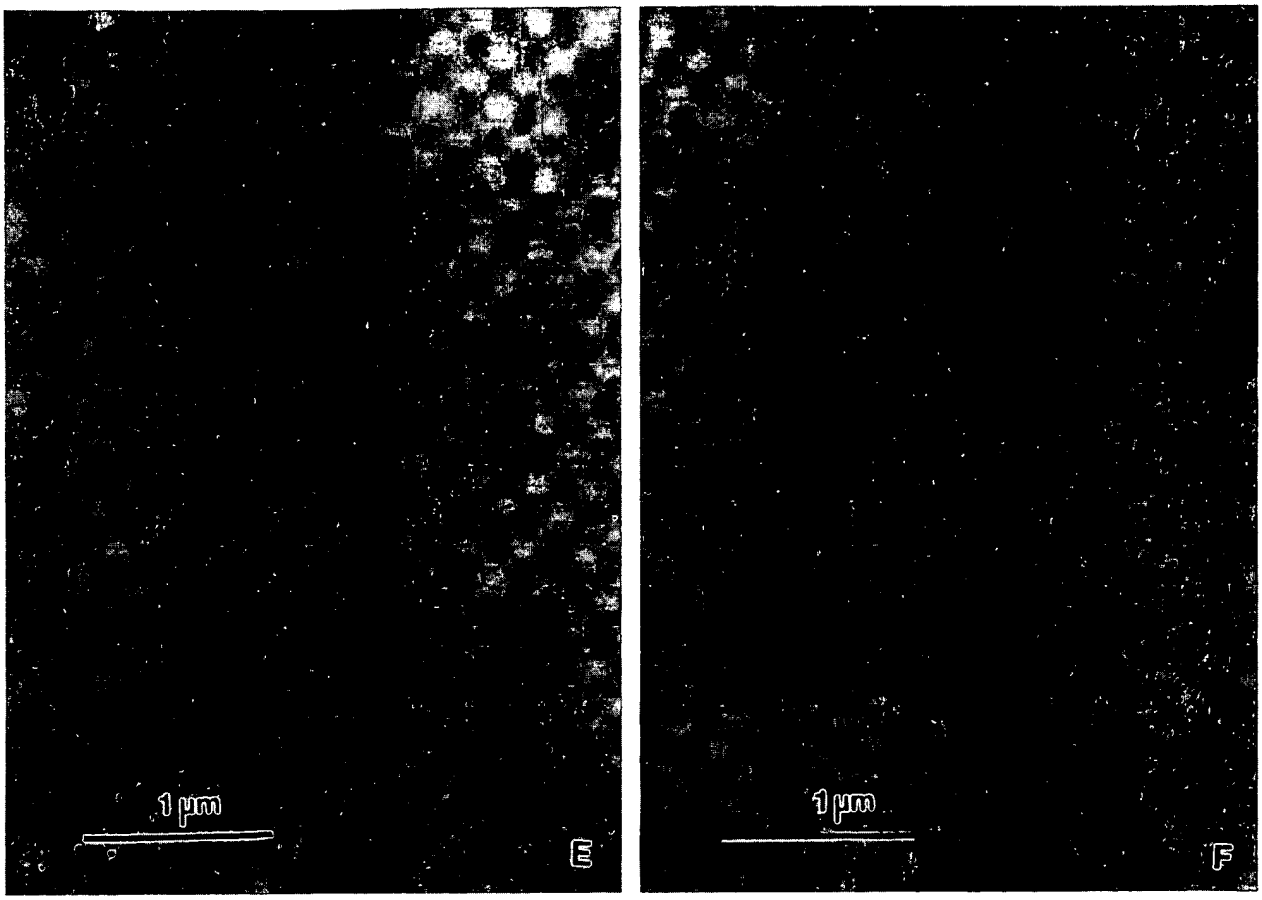

FIG 1. This and facing page. Photomicrographs. A, Localization of insulin-like growth factor (IGF-I) in synthetic smooth muscle cells (sSMC) in a restenotic atherectomy plaque. The secondary antibody is labeled with 15 nm colloidal gold. IGF-I localizes throughout the cytoplasm in endoplasmic reticulum–derived vesicles (arrowheads) and on cell processes. Original magnification, ×25 000. B, Control for specificity of IGF-I labeling. Comparable area in a section adjacent to that shown in Fig 1A that was reacted with IGF-I antibody absorbed with recombinant human IGF-I. Original magnification, ×25 000. C, Localization of IGF-I in a de novo plaque in the extracellular matrix (ECM) around contractile smooth muscle cells (cSMCs). The secondary antibody is labeled with 10 nm colloidal gold. IGF-I localizes predominantly in the ECM (arrowheads). Original magnification, ×25 000. D, Localization of IGF-I in sSMC in the same section as Fig 1D. There is increased localization in this sSMC compared with the cSMC. Original magnification, ×25 000. E, Localization of IGF-I in SMC of contractile phenotype from a coronary artery of a healthy individual. Original magnification, ×25 000. F, Control for specificity of IGF-I labeling. Comparable area in a parallel section to Fig 1E that was reacted with IGF-I antibody absorbed with recombinant human IGF-I. Original magnification, ×25 000.

tem. Insulin also increased the abundance of IGFBP-4. Exposure of SMCs to either PDGF, TGF-β, or b-FGF did not affect levels of either IGFBP-2 or IGFBP-4. Addition of IGFBP-4 to SMC cultures containing IGF-I had no effect on thymidine incorporation, whereas addition of IGFBP-4 to human fibroblasts with IGF-I resulted in near-complete inhibition of IGF-I–stimulated DNA synthesis.[29] Taken together, these studjes suggest that the factors that regulate IGFBPs vary and that this differential regulation may be an important mechanism by which SMC growth is controlled.

Our immunocytochemical studies and in vitro studies provide a firm basis for using somatostatin analogues for inhibition of SMC proliferation in accelerated atherogenesis. They also establish that somatostatin analogues could be more effective in inhibiting proliferation of rapidly proliferating SMCs rather than quiescent SMCs.

Our in vitro studies have shown that IGF-I stimulates human coronary SMC proliferation in a dose- and time-dependent manner. IGF-I and PDGF act synergistically to stimulate SMC proliferation. IGF-I has been called a progression factor; ie, when quiescent cells are exposed to mitogens like PDGF, they become competent to replicate but cannot proceed through the cell cycle without a progression factor like IGF-I. Both growth factors increase the level of c-*myc* RNA in SMCs,[27] and the translated product is a DNA-binding protein associated with cellular growth that regulates the entry of cells into the S-phase of the cell cycle.

Somatostatin and its analogues octreotide and angiopeptin inhibit cellular proliferation in a wide variety of tumors. Protein tyrosine phosphorylation plays a crucial role in the cellular regulation of proliferation, differentiation, and transformation and is controlled by two sets of enzymes: protein tyrosine kinases (PTKs) and PTPases. Various PTPases have been shown to dephosphorylate the phosphorylated form of the insulin receptor in vitro, suggesting that PTPases can control signal transduction mediated by PTKs. Following IGF-I, b-FGF, or PDGF binding to their receptors, autophosphorylation of the tyrosine kinase domain occurs and activates PTK to phosphorylate exogenous proteins. Autophosphoryla-

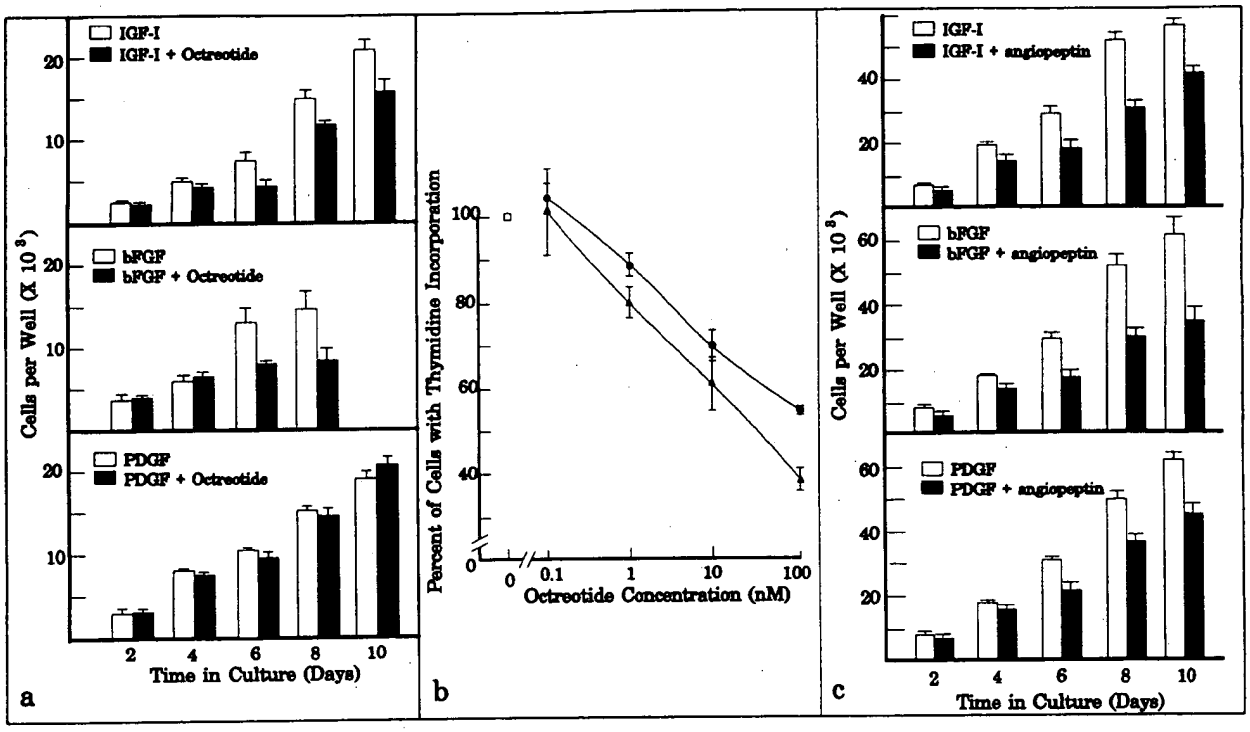

FIG 2. a, Bar graphs of growth factor–induced proliferation and octreotide inhibition of cultured human coronary smooth muscle cells (SMCs) grown for 8 to 10 days. Top, Inhibition of insulin-like growth factor (IGF-I)–induced proliferation by 10 nM octreotide (closed bars) versus IGF-I alone (open bars). Middle, Inhibition of basic fibroblast growth factor (b-FGF)–induced proliferation by 10 nM octreotide (closed bars) versus b-FGF alone (open bars). Bottom, Platelet-derived growth factor (PDGF) stimulation of proliferation (open bars) versus cells exposed to 10 nM PDGF and octreotide (closed bars). These data represent the mean±SEM cell count of quadruplicate wells. b, Plot of reduction in [^{3}H]thymidine uptake by cultured SMCs in response to growth factors and octreotide exposure. Cells were exposed to serial dilutions of octreotide along with either 10 nM IGF-I (●) or 10 nM b-FGF (▲) with 5 μCi [^{3}H]thymidine for 18 hours. Each point is the mean±SEM trichloroacetic acid precipitable counts for triplicate wells. The data are expressed as a percent of basal (100%) incorporation, ie, cells not exposed to octreotide (□). c, Bar graphs of growth factor–induced proliferation and angiopeptin inhibition of cultured human coronary SMCs. Top, Inhibition of IGF-I–induced proliferation by 30 nM angiopeptin (closed bars) versus IGF-I alone (open bars). Middle, Inhibition of b-FGF–induced proliferation by 30 nM angiopeptin (closed bars) versus b-FGF alone (open bars). Bottom, Inhibition of PDGF-induced proliferation by 30 nM angiopeptin (closed bars) versus PDGF alone (open bars). These data represent the mean±SEM cell count of quadruplicate wells.

tion renders PTK constituitively active, even when growth factor is subsequently removed from the binding site. Consequently, dephosphorylation, and not merely dissociation of the growth factor, is required to terminate PTK activity. In addition to the state of tyrosine autophosphorylation of each growth factor receptor, its degree of activation in vivo will depend on the relative activities of the PTPases involved in dephosphorylation.[30] Somatostatin analogues stimulate PTPases, which then inactivate the mitogenic potential of each of these growth factors.

In this in vitro study, we demonstrate that IGF-I, b-FGF, and PDGF stimulate DNA synthesis and cell proliferation in human coronary SMCs. The stimulating effect of IGF-I and b-FGF is blocked by the somatostatin analogue octreotide, and this inhibition occurs at a low concentration (10 nmol/L) of octreotide. The effective dose is comparable to that used in studies of other cell types. Octreotide also exerted a dose-dependent inhibition of thymidine incorporation in human coronary SMCs. Angiopeptin demonstrates a similar effect but also blocks the effect of PDGF on SMC proliferation. The reason for this is not entirely clear but may be related to differences in each analogue's ability to stimulate PTPases or differences in the PTPases that modulate these three growth factors.

Although antibodies to IGF-I and b-FGF inhibited migration induced by these growth factors, the somatostatin analogues did not. This may be due to the requirement of this assay that a single-cell suspension of SMC be prepared, as only single cells can migrate through the pores of the filters used for these studies. This necessitates aggressive trypsinization. Somatostatin receptors may be particularly sensitive to trypsin treatment, and the 12-hour duration of the migration assay may not permit adequate recovery of the somatostatin receptor. The proliferation assays, in contrast, were performed over 10 days, giving ample time for the receptors to recover or regenerate. For these reasons, the modified Boyden chamber assay may not adequately assess the effect of somatostatin analogues on in vitro SMC migration.

All three growth factors considered in this study have been shown to be produced by SMCs.[27,31-33] The in vivo mitogenic potential of these growth factors for SMCs is complex. Local cell injury, caused by interventional techniques, is required for release of b-FGF, as b-FGF is matrix bound. b-FGF can be released by plasminogen activators that are synthesized by cells several days after balloon injury.[34] Although additional IGF-I localization in restenotic lesions remains to be performed, our results in the de novo lesions support the involvement of

IGF-I in the pathogenesis of atherosclerosis. Local production of IGF-I by SMCs could facilitate SMC proliferation, thus contributing to the cellularity commonly seen in restenotic lesions.

Although extensive studies in the literature are available using aortic SMCs, studies using human coronary SMCs are extremely limited. Our results support the individual stimulatory effects of PDGF, IGF-I, and b-FGF on human coronary artery. Our data provide the basis to suggest the use of somatostatin analogues in the clinical setting to modify the high incidence of restenosis observed after coronary interventions by reducing SMC proliferation induced by IGF-I, b-FGF, and PDGF.

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References

1. Topol EJ, and the CAVEAT Investigators. Results of the CAVEAT trial. American College of Cardiology, Scientific Session; March 13-16, 1993; Anaheim, Calif.
2. Margolis JR, Mehta S. Excimer laser coronary angioplasty. *Am J Cardiol.* 1992;69:3F-11F.
3. Teirstein PS, Warth DC, Haq N, Jenkins NS, McCowan LC, Aubanel-Reidel P, Morris N, Ginsberg R. High speed rotational coronary atherectomy for patients with diffuse coronary artery disease. *J Am Coll Cardiol.* 1991;18:1694-1701.
4. Austin GE, Ratliff NB, Hollman J, Tabei S, Phillips DF. Intimal proliferation of smooth muscle cells as an explanation for recurrent coronary artery stenosis after percutaneous transluminal coronary angioplasty. *J Am Coll Cardiol.* 1985;6:369-375.
5. Essed CF, Brand MVD, Becker AE. Transluminal coronary angioplasty and early restenosis: fibrocellular occlusion after wall laceration. *Br Heart J.* 1983;49:393-396.
6. Ohara T, Kodama K, Mishima M, Nanto S, Hirayama A, Yutani C. Ultrastructural findings of proliferating and migrating smooth muscle cells at the site of percutaneous transluminal coronary angioplasty. *J Am Coll Cardiol.* 1988;11:131A.
7. Foegh ML. Chronic rejection-graft arteriosclerosis. *Transplant Proc.* 1990;22:119-122.
8. Raines E, Ross R. Mechanisms of plaque formation: cellular changes and possible role of growth-regulatory molecules. *Atheroscler Rev.* 1991;23:143-152.
9. Klagsbrun M, Edelman ER. Biological and biochemical properties of fibroblast growth factors: implications for the pathogenesis of atherosclerosis. *Arteriosclerosis.* 1989;9:269-278.
10. Grant MB, Caballero S, Wargovich TJ. Inhibition of smooth muscle cell proliferation by the somatostatin analogue, octreotide. *Endocrine Soc.* 1992:191A.
11. Nikol S, Isner JM, Pickering JG, Kearney M, Leclerc G, Weir L. Expression of transforming growth factor β1 is increased in human vascular restenosis lesions. *J Clin Invest.* 1992;90:1582-1592.
12. Rakugi H, Gibbons G, Wang D, Billingham M, Vershave K, Ferguson J, Angellini P, Hogan P, Mussumy A, Buja LM, Clubb F, McAllister H, Willerson J, Dzau V. Expression of transforming growth factor beta 1 in human atherectomy specimens from primary and restenotic coronary artery lesions. *J Am Coll Cardiol.* 1992;19:329A.
13. Yarden Y, Ullrich A. Growth factor receptor tyrosine kinases. *Annu Rev Biochem.* 1988;57:443-478.
14. Cool DE, Andreassen PR, Tonks NK, Krebs EG, Fischer EH, Morgolis RL. Cytokinetic failure and asynchronous nuclear division in BHK cells overexpressing a truncated protein-tyrosine-phosphatase. *Proc Natl Acad Sci U S A.* 1992;89:5422-5426.
15. Parmar H, Bogden A, Mollard M, de Rouge B, Phillips RH, Lightman SL. Somatostatin and somatostatin analogues in oncology. *Cancer Treat Rev.* 1989;16:95-115.
16. Merimee TJ. A follow-up study of vascular diabetes in growth hormone deficient dwarfs with diabetes. *N Engl J Med.* 1978;298:1217-1222.
17. Martins JB, Kerber RE, Sherman BM, Marcus ML, Ehrhardt JC. Cardiac size and function in acromegaly. *Circulation.* 1977;56:863-869.
18. Lundergan CF, Foegh ML, Ramwell PW. Peptide inhibition of myointimal proliferation by angiopeptin, a somatostatin analogue. *J Am Coll Cardiol.* 1991;17:132B-136B.
19. Tiell ML, Stemerman MB, Spaet TH. The influence of the pituitary on arterial intimal proliferation in the rat. *Circ Res.* 1978;42:644-649.
20. Santoian EC, Schneider JE, Gravanis MB, Foegh M, Tarazona N, Cipolla GD, King SB. Angiopeptin inhibits intimal hyperplasia after angioplasty in porcine coronary arteries. *Circulation.* 1993;88:11-14.
21. Thyberg J, Hedlin U, Sjolund M, Palmberg L, Bottger BA. Regulation of differentiated properties and proliferation of smooth muscle cells. *Arteriosclerosis.* 1990;10:966-990.
22. Clowes AW, Clowes MM, Kocher O, Ropraz P, Choponnier C, Gabbian G. Arterial smooth muscle cells in vivo: relationship between actin isoform expression and mitogenesis and their modulation by heparin. *J Cell Biol.* 1988;107:1339-1345.
23. Grant MB, Khaw PT, Schultz GS, Adams JL, Shimizu RW. Effects of epidermal growth factor, fibroblast growth factor and transforming growth factor-β on corneal cell chemotaxis. *Invest Ophthalmol Vis Sci.* 1992;33:3292-3301.
24. Banskota NK, Carpentier JL, King GL. Processing and release of insulin and insulin-like growth factor I by macro- and microvascular endothelial cells. *Endocrinology.* 1986;119:1904-1913.
25. Hansson H-A, Brandsten C, Lossing C, Petruson K. Transient expression of insulin-like growth factor I immunoreactivity by vascular cells during angiogenesis. *Exp Mol Pathol.* 1989;50:125-138.
26. Libby P, Schwartz D, Brogi E, Tanaka H, Clinton K. A cascade model for restenosis: a special case of atherosclerosis progression. *Circulation.* 1992;86(suppl III):III-47-III-52.
27. Banskota NK, Taub R, Zeller K, King GL. Insulin, insulin-like growth factor I and platelet derived growth factor interact additively in the induction of the protooncogene c-myc and cellular proliferation in cultured bovine aortic smooth muscle cells. *Mol Endocrinol.* 1989;3:1183-1190.23.
28. Elgin RG, Busby WH Jr, Clemmons DR. An insulin-like growth factor (IGF) binding protein enhances the biological response to IGF-I. *Proc Natl Acad Sci U S A.* 1987;84:3254-3258.
29. Cohick WS, Gockerman A. Regulation of IGF binding protein synthesis and bioavailability in cultured vascular smooth muscle cells. *Endocrine Soc.* 1993:1484A.
30. Colas B, Cambillau C, Buscail L, Zeggari M, Esteve JP, Lautre V, Thomas F, Vaysse N, Susini C. Stimulation of a membrane tyrosine phosphatase activity by somatostatin analogues in rat pancreatic acinar cells. *Eur J Biochem.* 1992;207:1017-1024.
31. Libby P, Warner SJC, Salomon RN, Birinyi LK. Production of platelet-derived growth factor-like mitogen by smooth-muscle cells from human atheroma. *N Engl J Med.* 1988;318:1493-1496.
32. Winkles JA, Friesel R, Burgess WH, Howk R, Mehlman T, Weinstin R, Maciag T. Human vascular smooth muscle cells both express and respond to heparin-binding growth factor I (endothelial cell growth factor). *Proc Natl Acad Sci U S A.* 1987;84:7124-7128.
33. Cercek B, Fishbein MC, Forrester JS, Helfant RH, Fagin JA. Induction of insulin-like growth factor I messenger RNA in rat aorta after balloon denudation. *Circ Res.* 1990;66:1755-1760.
34. Kirschstein W, Simianer S, Dempfle CE, Keller H, Stegaru B, Rentrop P, Heene DL. Impaired fibrinolytic capacity and tissue plasminogen activator release in patients with restenosis after percutaneous transluminal coronary angioplasty (PTCA). *Thromb Haemost.* 1989;62:772-775.